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# Electrochemical study of lincomycin on a multi-wall carbon nanotubes modified glassy carbon electrode and its determination in tablets

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**Abstract**

Multi-wall carbon nanotubes (MWNTs) were dispersed in dihexadecylphosphate (DHP), sodium dodecylbenzenesulfonate (SDBS), and Nafion to give a homogeneous and stable suspension, respectively. MWNTs film in different solvents was fabricated onto a glassy carbon electrode (GCE) with an easy and convenient method. It was found that on MWNTs-DHP film lincomycin exhibited a well-defined oxidation peak. The electrochemical behavior of lincomycin at coated GCE was investigated by cyclic voltammetry and chronocoulometry. The MWNTs-DHP film modified glassy carbon electrode shows obvious electrocatalytic activity to the oxidation of lincomycin, since it greatly enhances the oxidation peak current of lincomycin as well as lowers its oxidation overpotential. Based on this, a very sensitive and simple voltammetric method was developed for the measurement of lincomycin. A sensitive linear voltammetric response for lincomycin was obtained in the concentration range of $4.5 \times 10^{-7}$ to $1.5 \times 10^{-4}$ mol/l, and the detection limit is $2.0 \times 10^{-7}$ mol/l using linear sweep voltammetry. Compared with other methods, this proposed method possesses many advantages such as very low detection limit, fast response, low cost and simplicity. The practical application was demonstrated to determine lincomycin in tablets with good result.


*Keywords:* Lincomycin; Dihexadecylphosphate; Multi-wall carbon nanotubes; Electrochemical method

## 1. Introduction

Lincomycin (Scheme 1) is a well-established antibiotic drug used in human and veterinary medicine. It is effective primarily against gram-positive pathogens and plays its role by binding to the 50S subunit of the ribosome, thus inhibiting bacterial protein synthesis. Lincomycin was widely applied in medicine field, thus it is important to develop an effective method to determine lincomycin. Currently spectrometry [1], liquid chromatography [2-4] and capillary electrophoresis [5,6] are often used to determine lincomycin. Direct electrochemical method to determine lincomycin on MWNTs modified electrode has not yet been reported.

Carbon nanotubes are molecular-scale wires with high electrical conductivity, high chemical stability, and extremely high mechanical strength and modulus [7]. The subtle electronic behavior of CNTs reveals that they have the ability to promote electron-transfer reaction when used as electrode materials. In this work, MWNTs were dispersed in dihexadecylphosphate (DHP), sodium dodecylbenzenesulfonate (SDBS), and Nafion, respectively. MWNTs film was achieved on GCE surface via solvent evaporation. The electrochemical behavior of lincomycin was investigated on MWNTs-DHP, MWNTs-SDBS, and MWNTs-Nafion film coated GCE, respectively. It was found that on MWNTs-DHP composite film lincomycin exhibited a well-defined oxidation peak. The electrochemical behavior of lincomycin on MWNTs-DHP coated GCE was studied by cyclic voltammetry and chronocoulometry. Based on the electrochemical results, the MWNTs-DHP coated GCE was proposed to determine lincomycin. After optimizing the experimental parameters, a linear sweep voltammetric method was developed for the direct determination of lincomycin. Compared with other published methods, this newly proposed method possesses many advantages such as very low detection limit, fast response, low cost and simplicity. It was successfully applied for the determination of lincomycin in tablets.

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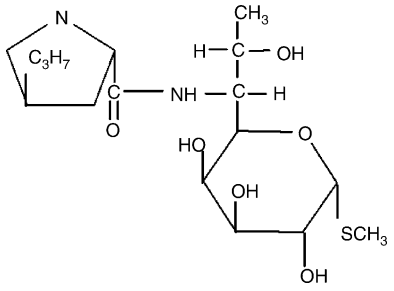


Scheme 1. Structural formula of lincomycin.

## 2. Experimental

### 2.1. Reagents and apparatus

Lincomycin was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Its stock solution (1 mg/ml, $2.5 \times 10^{-3}$ mol/l) was prepared with methanol and stored in a refrigerator at 4 °C. Sodium dodecylbenzenesulfonate (SDBS) was bought from Shanghai Chemical Reagents Co. Ltd. (China). DHP and Nafion (5% in ethanol) were purchased from Fluka. Clear vesicle dispersions of DHP (5 mM) in water were obtained by overnight ultrasonication. All other chemicals were of analytical grade and were used without further purification. Doubly-distilled water was used throughout and the supporting electrolyte was usually phosphate buffer containing 0.06 M $Na_2HPO_4$ and $NaH_2PO_4$. The Commercially available drug tablets were purchased from the market.

The multi-wall carbon nanotubes (obtained from the Institute of Nanometer Materials, Central China Normal University, China) were synthesized by a catalytic pyrolysis method and purified with concentrated $HNO_3$ [8].

All electrochemical measurements were performed with a computer controlled Model CHI830A electrochemical analyzer (Chenghua Instrument Co., Shanghai, China). A three-electrode cell was employed with a platinum wire as counter electrode, a saturated calomel electrode (SCE) as reference electrode and a multi-wall carbon nanotube modified glassy electrode as working electrode. All potentials were quoted with respect to SCE.

### 2.2. Preparation of the MWNTs modified GCE

A glassy carbon electrode of 3-mm diameter was used. It was polished with 0.3 and 0.05 μm alumina slurry (CH instrument, Inc.) in sequence, then sonicated in ethanol and doubly distilled water, respectively.

Multi-wall carbon nanotube (MWNTs, 1 mg) was added into 1 ml clear vesicle aqueous dispersions of DHP (5 mM), 1 ml SDBS ($1 \times 10^{-2}$ mol/l in water) and 1 ml Nafion (0.1% in ethanol) solution, respectively. A well-dispersed of MWNTs-DHP, MWNTs-SDBS and MWNTs-Nafion solutions were obtained by ultrasonication for 5 min. The GCE was coated by dropping 5 μl dispersions of MWNTs and dried under infrared lamp in the air. The DHP-film coated GCE was prepared by the same procedure as explained above, but without MWNT. The freshly prepared MWNTs-modified electrodes were activated in 0.06 M phosphate buffer (pH 7.0) by cyclic scans from −0.2 to 1.2 V successively and continued until the shape of the cyclic curves no longer changed. After each measurement, the electrode was refreshed by potential scans mentioned above.

### 2.3. Procedures

The supporting electrolyte for electrochemical measurements was 0.06 M phosphate buffer. Certain volume of standard solution of lincomycin was added into the 10 ml cell containing phosphate buffer. Then the mixture solution was stirred for 2 min and kept unstirred for 20 s at open circuit. The oxidation peak of lincomycin at 0.85 V was recorded in the potential range from −0.2 to 1.2 V.

## 3. Results and discussion

### 3.1. Electrochemical behavior of lincomycin at MWNTs modified GCE

Fig. 1 shows the cyclic voltammograms of lincomycin at MWNTs-DHP (a), MWNTs-SDBS (b), and MWNTs-Nafion (c) modified GCE, respectively. It is obvious that at MWNTs-DHP film lincomycin exhibits a well-defined oxidation peak at 0.85 V. The peak current of lincomycin at MWNTs-SDBS film is much higher, but the oxidation peak is not well-defined and the peak potential is much higher than that at MWNTs-DHP film. At MWNTs-Nafion film lincomycin displays no obvious oxidation peak. The experimental results demonstrated that surfactants had influence on the electrode process of lincomycin. It confirms the conclusion that surfactants can alter the overvoltage of the electrode and influence the electron transfer rate [9,10]. MWNTs-DHP composite film provided a good platform for lincomycin to take electrode process, and DHP possessed

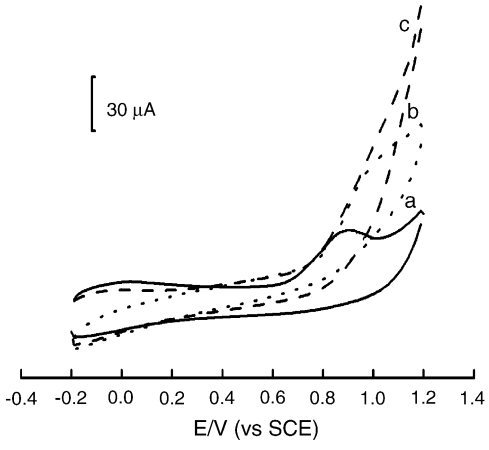


Fig. 1. Cyclic voltammograms of $2 \times 10^{-6}$ mol/l lincomycin at MWNTs-DHP coated GCE (a); MWNTs-SDBS coated GCE (b); MWNTs-Nafion coated GCE (c) with $t_{acc}$ = 2 min in 0.06 M phosphate buffer (pH 7.0). Scan rate is 100 mV/s.

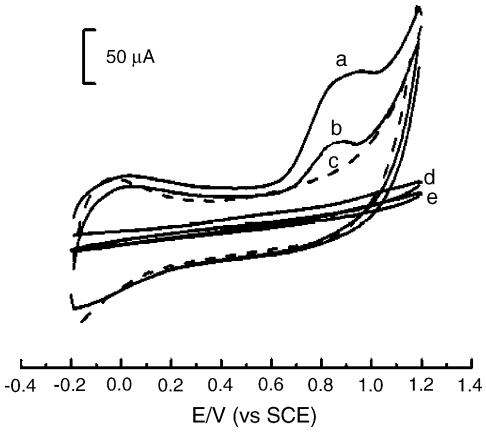


Fig. 2. Cyclic voltammograms of MWNTs-DHP coated GCE in phosphate buffer (pH 7.0) in the absence of lincomycin (c); MWNTs-DHP coated GCE in phosphate buffer (pH 7.0) in the presence of $2 \times 10^{-6}$ mol/l with $t_{acc} = 2$ min: first cycle (a); third cycle (b); DHP coated GCE in phosphate buffer (pH 7.0) in the presence of $2 \times 10^{-6}$ mol/l with $t_{acc} = 2$ min (d); Bare GCE in phosphate buffer (pH 7.0) in the presence of $2 \times 10^{-6}$ mol/l with $t_{acc} = 2$ min (e). Scan rate is 100 mV/s.

the property to form stable films on GCE, thus MWNTs-DHP composite film was chosen to investigate the electrochemical behavior of lincomycin in our work.

The cyclic voltammograms of a MWNT-modified GCE in phosphate buffer at pH 7.0 with and without of lincomycin are illustrated in Fig. 2. In the potential range from −0.2 to 1.2 V, there are no observable redox peaks for a MWNT-modified GCE (Fig. 2c). However, upon addition of $2 \times 10^{-6}$ mol/l lincomycin, a well-defined oxidation peak appears at 0.85 V (Fig. 2a). On the reverse potential scan from 1.2 to −0.2 V, there is no corresponding reduction peak observed for lincomycin. Moreover, the oxidation peak current of lincomycin decreases remarkably during the successive cyclic potential sweeps (Fig. 2b). After the third cyclic voltammetric sweep at scan rate of 100 mV/s, the peak current maintains almost unchangeable. This phenomenon may be caused by the fact that the adsorption of lincomycin occurs at the surface of the electrode.

In order to illustrate the electrocatalytic effect of MWNT toward lincomycin, the electrochemical properties of lincomycin at bare GCE and DHP-modified GCE were studied using cyclic voltammetry, and the results shown in Fig. 2e and d. At bare GCE and DHP-modified GCE, $2 \times 10^{-6}$ mol/l lincomycin yields a small oxidation peak at 1.0 V in phosphate buffer at pH 7.0 (curve d and e). However, the oxidation peak current of lincomycin at the MWNT-modified GCE increases significantly and the peak potential shifts towards less positive potentials at 0.85 V (curve a) in comparison with that at bare GCE. The remarkable peak current enhancement and the decrease of oxidation overpotential undoubtedly testify the conclusion that nanotubes are effective electrocatalysts [11,12]. MWNT-modified GCE greatly improves the sensitivity of the determination of lincomycin on account of the unusual structure and properties of MWNT (such as very large specific area, strong adsorptive ability and subtle electronic properties).

### 3.2. Effects of scan rates and pH on the oxidation of lincomycin at MWNTs-DHP modified GCE

The effect of potential scan rate, $\nu$, on the peak current and peak potential of lincomycin were evaluated by cyclic voltammetry from −0.2 to 1.2 V. The result showed that from 10 to 500 mV/s, the peak current was proportional to the scan rate (the inset in Fig. 3), suggesting that an adsorption-controlled process was involved in the oxidation of lincomycin.

Fig. 2 shows that the electrode reaction of lincomycin is an irreversible surface reaction. For the irreversible surface electrochemical reaction, the relationship between the peak potential $E_p$ and the scan rate $\nu$ in the cyclic voltammogram have been expressed in Eq. (1) by Laviron [13]

$$E_p = E^{0'} + (RT/\alpha nF) \ln (RTk_s/\alpha nF) - (RT/\alpha nF) \ln \nu \quad (1)$$

where $\alpha$ is the transfer coefficient, $k_s$, the standard rate constant of the surface reaction, and $E^{0'}$ is the formal potential. According to Eq. (1), the plot of $E_p \sim \ln \nu$ is linear with a slope allowing $\alpha n$ to be determined. From slope of the $E_p \sim \nu$ plot in Fig. 3 (0.05278), $\alpha n = 0.46$ was calculated. In most systems $\alpha$ turns out to lie between 0.3 and 0.7, and it can usually be approximated by 0.5 in the absence of actual measurements [14]. These results, therefore, demonstrate that one electron is involved in the oxidation of lincomycin.

The influence of pH on the oxidation of lincomycin was studied in the pH range of 4.5–9.5. The effect of pH on the oxidation peak current is shown in the inset of Fig. 4. The peak current reaches the maximum at pH 7.0, and the pH value is similar to physiological pH, so this pH value was adopted in the following experiment. Fig. 4 shows the $E_p \sim$ pH relationship. $E_p$ shifts less positively with the increase of pH from 4.5 to 8. The linear regions of the $E_p$ − pH plot intersect at about 8. At pH >8, the $E_p$ is almost pH-independent. The anodic peak potential of lincomycin shifted linearly towards less positive values with increasing the pH by 0.0963 V/pH. Based on Eq. (2), the number of hydrogen ion taking part in the electrode reactions was estimated as 1.

In aqueous solution, for the reaction

$$Ox + ne + qH^+ = Red$$

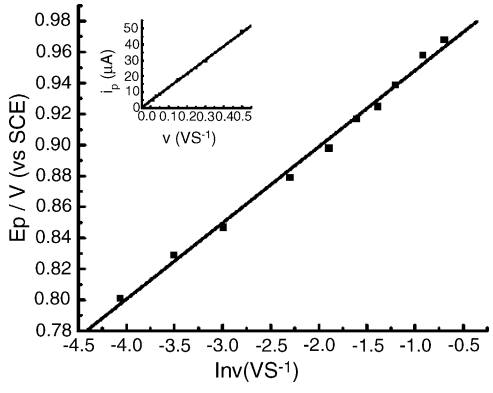


Fig. 3. Dependence of peak potential, $E_p$, on the scan rates, ln $\nu$. Inset: effects of scan rates on peak currents $i_{pa}$.

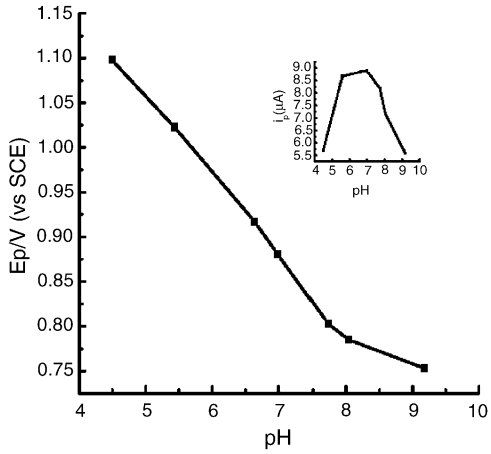


Fig. 4. Effect of pH on peak potential for $2 \times 10^{-6}$ mol/l lincomycin in phosphate buffer by linear sweep voltammetry at MWNTs-DHP coated GCE. Inset: variation of peak current, $i_p$ (μA), with different pH.

the $E_p$ value of the reaction is estimated by the following equation [15]:

$$E_p = E^{0'} + 2.303(qRT/\alpha nF) \log H^+ \quad (2)$$

where $E^{0'}$ is the formal potential, $q$ is the number of the protons involved in the reaction and $\alpha n$ was determined as 0.46 (see above). Other terms have their usual meanings.

### 3.3. Adsorption of lincomycin on the MWNTs-DHP film measured by single-potential step chronocoulometry

The chronocoulometric method was applied to determine the diffusion coefficient $D$ and $Q_{ads}$ of lincomycin on the MWNTs-modified electrode, according to the formula given by Anson [16]

$$Q = 2nFAC(Dt)^{1/2}/\pi^{1/2} + Q_{dl} + Q_{ads} \quad (3)$$

$Q_{dl}$ is double-layer charge, $Q_{ads}$ the Faradaic charge due to the oxidation of adsorbed lincomycin. $Q_{dl}$ is assumed not changed in the presence and absence of lincomycin in our work. The plot of $Q$ versus $t^{1/2}$ should be linear. From the slope and intercept, the values of $D$ and $Q_{ads}$ can be obtained. In our experiment, the plot of net charge (point by point background subtraction) against $t^{1/2}$ shows straight line (Fig. 5). The values of the slope and $Q_{ads}$ in Fig. 5 are $8.1 \times 10^{-5}$ and $3.17 \times 10^{-6}$ C, respectively. As the number of electron involved in the oxidation of lincomycin is 1 and $A = 7.2 \times 10^{-2}$ cm$^2$, $C = 3.0 \times 10^{-6}$ mol/l, it is calculated that $D = 3.76 \times 10^{-6}$ cm$^2$/s. The surface concentration, $\Gamma^s$, can be obtained by Eq. (4) as $5.32 \times 10^{-10}$mol/cm$^2$.

$$\Gamma^s = \frac{Q_{ads}}{nFA} \quad (4)$$

#### 3.3.1. Analytical application

*3.3.1.1. Optimization of experimental parameters.* The relationship between the amount of MWNTs-DHP dispersion on the GCE surface and the oxidation peak current of lincomyin were tested and the results are shown in Fig. 6. The oxidation peak

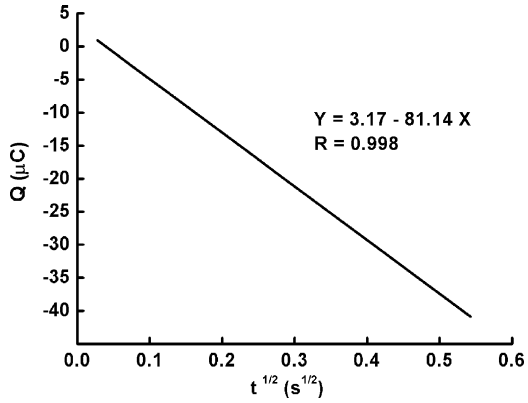


Fig. 5. The plot of $Q \sim t^{1/2}$ for $2 \times 10^{-6}$ mol/l lincomycin at MWNTs-DHP coated GCE by background subtraction. $t_{acc} = 2$ min; intial potential: −0.2 V; final potential: 1.2 V.

current is very high when using 5 μL of 1 mg/l MWNTs-DHP dispersion to coat GCE. On further increasing the amounts of MWNTs-DHP dispersion, the peak currents begin to decrease gradually. However, the amounts of MWNTs-DHP dispersion exceed 7.5 μl, the peak currents greatly decrease. It is mainly due to the blocking behavior of DHP.

Effects of accumulation potential and accumulation time on the oxidation of lincomycin were also investigated. The peak currents were larger at an open circuit accumulation than the closed circuit accumulation. At an open circuit it is beneficial for the adsorption of lincomycin onto the MWNTs-DHP surface. The peak current increased with the accumulation time because of the more adsorption. However, the peak current remained almost unchanged because of the saturate adsorption of lincomycin on MWNTs-DHP film when the accumulation was longer than 2 min.

*3.3.1.2. Calibration graph.* Under optimized experimental parameters, the calibration curve was obtained in pH 7.0 phosphate buffer by linear sweep voltammetry (Fig. 7). The linear segment increases from $4.5 \times 10^{-7}$ to $1.5 \times 10^{-4}$ mol/l, ($r = 0.995$) with a regression equation of $i_p = 0.05 + 0.58 \times 10^6\, C$ ($r = 0.995$, $C$ in mol/l, $i_p$ in μA). It was found that this method can detect $2 \times 10^{-7}$ mol/l lincomycin after 2 min of accumu-

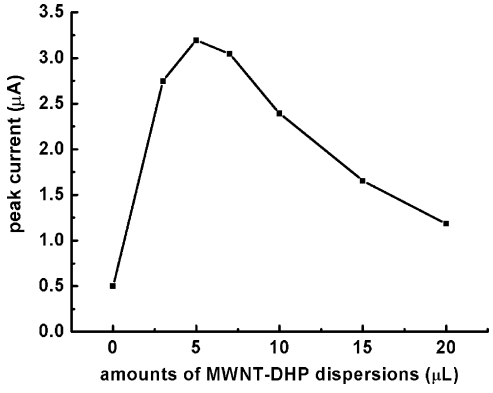


Fig. 6. Effects of 1 mg/l MWNTs-DHP suspensions on peak current of $2 \times 10^{-6}$ mol/l lincomycin in pH 7.0 phosphate buffer. Scan rate, 100 mV/s; $t_{acc} = 2$ min.

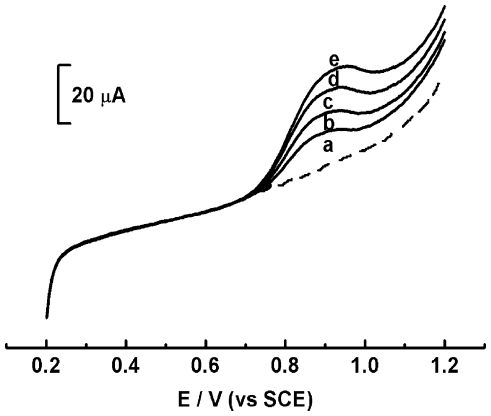


Fig. 7. Linear sweep voltammograms of lincomycin on MWNTs-DHP coated GCE in phosphate buffer (pH 7.0) in the presence of 0 mol/l (a); $2 \times 10^{-6}$ mol/l (b); $2.5 \times 10^{-6}$ mol/l (c); $3 \times 10^{-6}$ mol/l (d); $3.5 \times 10^{-6}$ mol/l (e) lincomycin with $t_{acc} = 2$ min. Scan rate, 100 mV/s.

lation. The relative standard deviation (R.S.D.) of 2.3% for $2 \times 10^{-6}$ mol/l lincomycin ($n = 7$) showed good reproducibility. The linearity and LOD for lincomycin by this electrochemical method was compared with other method, such as capillary electrophoresis [6] and liquid chromatography [4] and the results were listed in Table 1. It indicated that the presented method possessed the advantages of wide linearity range and very low detection limit for lincomycin.

The stability of the MWNT-modified GCE was evaluated by measuring the current responses at a fixed lincomycin concentration of $2 \times 10^{-6}$ mol/l over a period of 3 weeks. The MWNT-modified GCE was used daily and stored in the air. The experimental results indicated that the current responses deviated only 5.4%, revealing that the MWNT modified GCE fabricated by this method possesses long-term stability.

*3.3.1.3. Lincomycin assay in pharmaceutical formulations.* Twenty tablets were weighted accurately and crushed to a fine powder. Two-hundred and fifty milligrams of this powder was transferred to a 50 ml flask and was dissolved in 25 ml methanol. After sonication it was filtered. An aliquot of the filtrate was placed in a calibrated flask and diluted with pH 7.0 phosphate buffer.

The prepared solution from drug tablets was detected on MWNTs-DHP modified GCE by linear sweep voltammetry. The amount of lincomycin present in tablet was calculated from the calibration equation and the results are shown in Table 2. The relative standard deviation of 0.48–0.84% indicates the applicability of the proposed method. The effect of excipients on the voltammetric response of lincomycin was investigated. It was found that microcrystalline cellulose, hydroxypropylmethylcellulose, and lactose do not cause interferences.

Table 1
The linearity and LOD for lincomycin by various method

| Analyte | Linearity | LOD (μM) | Ref. |
|---|---|---|---|
| Lincomycin | $4.0 \times 10^{-5}$–$2.0 \times 10^{-3}$ mol/l | 6.7 | [6] |
| Lincomycin | 0.14–0.84 μg | – | [4] |
| Lincomycin | $4.5 \times 10^{-7}$–$1.5 \times 10^{-4}$ mol/l | 0.2 | This method |

Table 2
Determination of lincomycin in drug tablets by the proposed voltammetric method

| Sample no. | Lable claim (mg) | Found (mg)[a] | R.S.D. (%) |
|---|---|---|---|
| 1 | 250 | 247.9 | 0.84 |
| 2 | 250 | 251.2 | 0.48 |
| 3 | 250 | 248.1 | 0.76 |
| 4 | 250 | 251.6 | 0.64 |
| 5 | 250 | 250.4 | 0.16 |

[a] Average of seven determinations.

## 4. Conclusion

In this paper, an easy method to preparation a MWNTs-DHP composite film on the GCE surface is described. The electrochemical behavior of lincomycin on MWNTs-DHP film coated GCE was investigated by cyclic voltammetry and chronocoulometry. The MWNTs-DHP composite film provided a good platform to detect lincomycin, and it was applied for the determination of lincomycin in tablets with good result. The proposed method is a good alternative for the analytical determination of lincomycin because it is simple, fast and of low cost and it has sufficient precision, accuracy and sensitivity.

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